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Research review paper

Biodiesel from microalgae

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Abstract

Continued use of petroleum sourced fuels is now widely recognized as unsustainable because of depleting supplies and the contribution of these fuels to the accumulation of carbon dioxide in the environment. Renewable, carbon neutral, transport fuels are necessary for environmental and economic sustainability. Biodiesel derived from oil crops is a potential renewable and carbon neutral alternative to petroleum fuels. Unfortunately, biodiesel from oil crops, waste cooking oil and animal fat cannot realistically satisfy even a small fraction of the existing demand for transport fuels. As demonstrated here, microalgae appear to be the only source of renewable biodiesel that is capable of meeting the global demand for transport fuels. Like plants, microalgae use sunlight to produce oils but they do so more efficiently than crop plants. Oil productivity of many microalgae greatly exceeds the oil productivity of the best producing oil crops. Approaches for making microalgal biodiesel economically competitive with petrodiesel are discussed.
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Keywords: Biofuels; Biodiesel; Microalgae; Photobioreactors; Raceway ponds

Contents

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1. Introduction

Microalgae are sunlight-driven cell factories that convert carbon dioxide to potential biofuels, foods, feeds and high-value bioactives (Metting and Pyne, 1986; Schwartz, 1990; Kay, 1991; Shimizu, 1996, 2003; Borowitzka, 1999; Ghirardi et al., 2000; Akkerman et al., 2002; Banerjee et al., 2002; Melis, 2002; Lorenz and Cysewski, 2003; Metzger and Largeau, 2005; Singh et al., 2005; Spolaore et al., 2006; Walter et al., 2005). In addition, these photosynthetic microorganisms are useful in bioremediation applications (Mallick, 2002; Suresh and Ravishankar, 2004; Kalin et al., 2005; Munoz and Guieysse, 2006) and as nitrogen fixing biofertilizers Vaishampayan et al., 2001). This article focuses on microalgae as a potential source of biodiesel.

Microalgae can provide several different types of renewable biofuels. These include methane produced by anaerobic digestion of the algal biomass (Spolaore et al., 2006); biodiesel derived from microalgal oil (Roessler et al., 1994; Sawayama et al., 1995; Dunahay et al., 1996; Sheehan et al., 1998; Banerjee et al., 2002; Gavrilescu and Chisti, 2005); and photobiologically produced biohydrogen (Ghirardi et al., 2000; Akkerman et al., 2002; Melis, 2002; Fedorov et al., 2005; Kapdan and Kargi, 2006). The idea of using microalgae as a source of fuel is not new (Chisti, 1980–81; Nagle and Lemke, 1990; Sawayama et al., 1995), but it is now being taken seriously because of the escalating price of petroleum and, more significantly, the emerging concern about global warming that is associated with burning fossil fuels (Gavrilescu and Chisti, 2005).

Biodiesel is produced currently from plant and animal oils, but not from microalgae. This is likely to change as several companies are attempting to commercialize microalgal biodiesel. Biodiesel is a proven fuel. Technology for producing and using biodiesel has been known for more than 50 years (Knothe et al., 1997; Fukuda et al., 2001; Barnwal and Sharma, 2005; Demirbas, 2005; Van Gerpen, 2005; Felizardo et al., 2006; Kulkarni and Dalai, 2006; Meher et al., 2006). In the United States, biodiesel is produced mainly from soybeans. Other sources of commercial biodiesel include canola oil, animal fat, palm oil, corn oil, waste cooking oil (Felizardo et al., 2006; Kulkarni and Dalai, 2006), and jatropha oil (Barnwal and Sharma, 2005). The typically used process for commercial production of biodiesel is explained in Box 1. Any future production of biodiesel from microalgae is expected to use the same process. Production of methyl esters, or biodiesel, from microalgal oil has been demonstrated (Belarbi et al.,

Box 1

Biodiesel production

Parent oil used in making biodiesel consists of triglycerides (Fig. B1) in which three fatty acid molecules are esterified with a molecule of glycerol. In making biodiesel, triglycerides are reacted with methanol in a reaction known as transesterification or alcoholysis. Transestrification produces methyl esters of fatty acids, that are biodiesel, and glycerol (Fig. B1). The reaction occurs stepwise: triglycerides are first converted to diglycerides, then to monoglycerides and finally to glycerol.

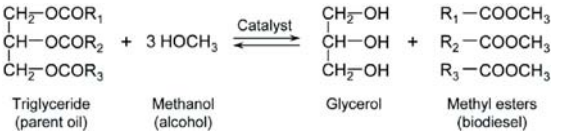

Fig. B1. Transesterification of oil to biodiesel. $R_{1–3}$ are hydrocarbon groups.

Transesterification requires 3 mol of alcohol for each mole of triglyceride to produce 1 mol of glycerol and 3 mol of methyl esters (Fig. B1). The reaction is an equilibrium. Industrial processes use 6 mol of methanol for each mole of triglyceride (Fukuda et al., 2001). This large excess of methanol ensures that the reaction is driven in the direction of methyl esters, i.e. towards biodiesel. Yield of methyl esters exceeds 98% on a weight basis (Fukuda et al., 2001).

Transesterification is catalyzed by acids, alkalis (Fukuda et al., 2001; Meher et al., 2006) and lipase enzymes (Sharma et al., 2001). Alkali-catalyzed transesterification is about 4000 times faster than the acid catalyzed reaction (Fukuda et al., 2001). Consequently, alkalis such as sodium and potassium hydroxide are commonly used as commercial catalysts at a concentration of about 1% by weight of oil. Alkoxides such as sodium methoxide are even better catalysts than sodium hydroxide and are being increasingly used. Use of lipases offers important advantages, but is not currently feasible because of the relatively high cost of the catalyst (Fukuda et al., 2001). Alkali-catalyzed transesterification is carried out at approximately 60 °C under atmospheric pressure, as methanol boils off at 65 °C at atmospheric pressure. Under these conditions, reaction takes about 90 min to complete. A higher temperature can be used in combination with higher pressure, but this is expensive. Methanol and oil do not mix, hence the reaction mixture contains two liquid phases. Other alcohols can be used, but methanol is the least expensive. To prevent yield loss

(continued on next page)

Box 1 (*continued*)

due to saponification reactions (i.e. soap formation), the oil and alcohol must be dry and the oil should have a minimum of free fatty acids. Biodiesel is recovered by repeated washing with water to remove glycerol and methanol.

2000) although the product was intended for pharmaceutical use.

2. Potential of microalgal biodiesel

Replacing all the transport fuel consumed in the United States with biodiesel will require 0.53 billion m^3 of biodiesel annually at the current rate of consumption. Oil crops, waste cooking oil and animal fat cannot realistically satisfy this demand. For example, meeting only half the existing U.S. transport fuel needs by biodiesel, would require unsustainably large cultivation areas for major oil crops. This is demonstrated in Table 1. Using the average oil yield per hectare from various crops, the cropping area needed to meet 50% of the U.S. transport fuel needs is calculated in column 3 (Table 1). In column 4 (Table 1) this area is expressed as a percentage of the total cropping area of the United States. If oil palm, a high-yielding oil crop can be grown, 24% of the total cropland will need to be devoted to its cultivation to meet only 50% of the transport fuel needs. Clearly, oil crops cannot significantly contribute to replacing petroleum derived liquid fuels in the foreseeable future. This scenario changes dramatically, if microalgae are used to produce biodiesel. Between 1 and 3% of the total U.S. cropping area would be sufficient for producing algal biomass that satisfies 50% of the transport fuel needs (Table 1). The microalgal oil yields given in Table 1 are based on experimentally demonstrated biomass productivity in photobioreactors, as discussed later in this article. Actual biodiesel yield per hectare is about 80% of the yield of the parent crop oil given in Table 1.

Table 1
Comparison of some sources of biodiesel

Crop	Oil yield (L/ha)	Land area needed (M ha) [a]	Percent of existing US cropping area [a]
Corn	172	1540	846
Soybean	446	594	326
Canola	1190	223	122
Jatropha	1892	140	77
Coconut	2689	99	54
Oil palm	5950	45	24
Microalgae [b]	136,900	2	1.1
Microalgae [c]	58,700	4.5	2.5

[a] For meeting 50% of all transport fuel needs of the United States.
[b] 70% oil (by wt) in biomass.
[c] 30% oil (by wt) in biomass.

Table 2
Oil content of some microalgae

Microalga	Oil content (% dry wt)
Botryococcus braunii	25–75
Chlorella sp.	28–32
Crypthecodinium cohnii	20
Cylindrotheca sp.	16–37
Dunaliella primolecta	23
Isochrysis sp.	25–33
Monallanthus salina	>20
Nannochloris sp.	20–35
Nannochloropsis sp.	31–68
Neochloris oleoabundans	35–54
Nitzschia sp.	45–47
Phaeodactylum tricornutum	20–30
Schizochytrium sp.	50–77
Tetraselmis sueica	15–23

In view of Table 1, microalgae appear to be the only source of biodiesel that has the potential to completely displace fossil diesel. Unlike other oil crops, microalgae grow extremely rapidly and many are exceedingly rich in oil. Microalgae commonly double their biomass within 24 h. Biomass doubling times during exponential growth are commonly as short as 3.5 h. Oil content in microalgae can exceed 80% by weight of dry biomass (Metting, 1996; Spolaore et al., 2006). Oil levels of 20–50% are quite common (Table 2). Oil productivity, that is the mass of oil produced per unit volume of the microalgal broth per day, depends on the algal growth rate and the oil content of the biomass. Microalgae with high oil productivities are desired for producing biodiesel.

Depending on species, microalgae produce many different kinds of lipids, hydrocarbons and other complex oils (Banerjee et al., 2002; Metzger and Largeau, 2005; Guschina and Harwood, 2006). Not all algal oils are satisfactory for making biodiesel, but suitable oils occur commonly. Using microalgae to produce biodiesel will not compromise production of food, fodder and other products derived from crops.

Potentially, instead of microalgae, oil producing heterotrophic microorganisms (Ratledge, 1993; Ratledge and Wynn, 2002) grown on a natural organic carbon source such as sugar, can be used to make biodiesel; however, heterotrophic production is not as efficient as using photosynthetic microalgae. This is because the renewable organic carbon sources required for growing heterotrophic microorganisms are produced ultimately by photosynthesis, usually in crop plants.

Production of algal oils requires an ability to inexpensively produce large quantities of oil-rich microalgal biomass.

3. Microalgal biomass production

Producing microalgal biomass is generally more expensive than growing crops. Photosynthetic growth requires light, carbon dioxide, water and inorganic salts. Temperature must remain generally within 20 to 30 °C. To minimize expense, biodiesel production must rely on freely available sunlight, despite daily and seasonal variations in light levels.

Growth medium must provide the inorganic elements that constitute the algal cell. Essential elements include nitrogen (N), phosphorus (P), iron and in some cases silicon. Minimal nutritional requirements can be estimated using the approximate molecular formula of the microalgal biomass, that is $CO_{0.48}H_{1.83}N_{0.11}P_{0.01}$. This formula is based on data presented by Grobbelaar (2004). Nutrients such as phosphorus must be supplied in significant excess because the phosphates added complex with metal ions, therefore, not all the added P is bioavailable. Sea water supplemented with commercial nitrate and phosphate fertilizers and a few other micronutrients is commonly used for growing marine microalgae (Molina Grima et al., 1999). Growth media are generally inexpensive.

Microalgal biomass contains approximately 50% carbon by dry weight (Sánchez Mirón et al., 2003). All of this carbon is typically derived from carbon dioxide. Producing 100 t of algal biomass fixes roughly 183 t of carbon dioxide. Carbon dioxide must be fed continually during daylight hours. Feeding controlled in response to signals from pH sensors minimizes loss of carbon dioxide and pH variations. Biodiesel production can potentially use some of the carbon dioxide that is released in power plants by burning fossil fuels (Sawayama et al., 1995; Yun et al., 1997). This carbon dioxide is often available at little or no cost.

Ideally, microalgal biodiesel would be carbon neutral, as all the power needed for producing and processing the algae would come from biodiesel itself and from methane produced by anaerobic digestion of biomass residue left behind after the oils has been extracted. Although microalgal biodiesel can be carbon neutral, it will not result in any net reduction in carbon dioxide that is accumulating as a consequence of burning of fossil fuels.

Large-scale production of microalgal biomass generally uses continuous culture during daylight. In this method of operation, fresh culture medium is fed at a constant rate and the same quantity of microalgal broth is withdrawn continuously (Molina Grima et al., 1999). Feeding ceases during the night, but the mixing of broth must continue to prevent settling of the biomass (Molina Grima et al., 1999). As much as 25% of the biomass produced during daylight, may be lost during the night because of respiration. The extent of this loss depends on the light level under which the biomass was grown, the growth temperature, and the temperature at night.

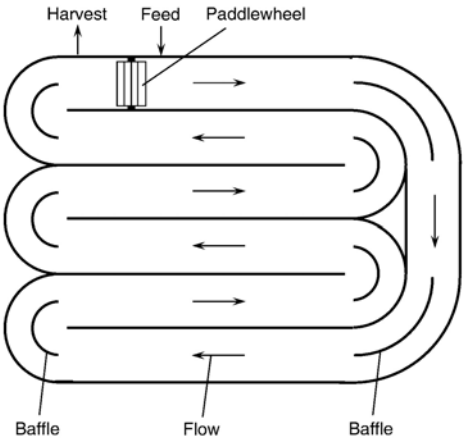

Fig. 1. Arial view of a raceway pond.

The only practicable methods of large-scale production of microalgae are raceway ponds (Terry and Raymond, 1985; Molina Grima, 1999) and tubular photobioreactors (Molina Grima et al., 1999; Tredici, 1999; Sánchez Mirón et al., 1999), as discussed next.

3.1. Raceway ponds

A raceway pond is made of a closed loop recirculation channel that is typically about 0.3 m deep (Fig. 1). Mixing and circulation are produced by a paddlewheel (Fig. 1). Flow is guided around bends by baffles placed in the flow channel. Raceway channels are built in concrete, or compacted earth, and may be lined with white plastic. During daylight, the culture is fed continuously in front of the paddlewheel where the flow begins (Fig. 1). Broth is harvested behind the paddlewheel, on completion of the circulation loop. The paddlewheel operates all the time to prevent sedimentation.

Raceway ponds for mass culture of microalgae have been used since the 1950s. Extensive experience exists on operation and engineering of raceways. The largest raceway-based biomass production facility occupies an area of 440,000 m^2 (Spolaore et al., 2006). This facility,

owned by Earthrise Nutritionals (www.earthrise.com), is used to produce cyanobacterial biomass for food.

In raceways, any cooling is achieved only by evaporation. Temperature fluctuates within a diurnal cycle and seasonally. Evaporative water loss can be significant. Because of significant losses to atmosphere, raceways use carbon dioxide much less efficiently than photobioreactors. Productivity is affected by contamination with unwanted algae and microorganisms that feed on algae. The biomass concentration remains low because raceways are poorly mixed and cannot sustain an optically dark zone. Raceway ponds and other open culture systems for producing microalgae are further discussed by Terry and Raymond (1985).

Production of microalgal biomass for making biodiesel has been extensively evaluated in raceway ponds in studies sponsored by the United States Department of Energy (Sheehan et al., 1998). Raceways are perceived to be less expensive than photobioreactors, because they cost less to build and operate. Although raceways are low-cost, they have a low biomass productivity compared with photobioreactors.

3.2. Photobioreactors

Unlike open raceways, photobioreactors permit essentially single-species culture of microalgae for prolonged durations. Photobioreactors have been successfully used for producing large quantities of microalgal biomass (Molina Grima et al., 1999; Tredici, 1999; Pulz, 2001; Carvalho et al., 2006).

A tubular photobioreactor consists of an array of straight transparent tubes that are usually made of plastic or glass. This tubular array, or the solar collector, is where the sunlight is captured (Fig. 2). The solar collector tubes are generally 0.1 m or less in diameter. Tube diameter is limited because light does not penetrate too deeply in the dense culture broth that is necessary for ensuring a high biomass productivity of the photobioreactor. Microalgal broth is circulated from a reservoir (i.e. the degassing column in Fig. 2) to the solar collector

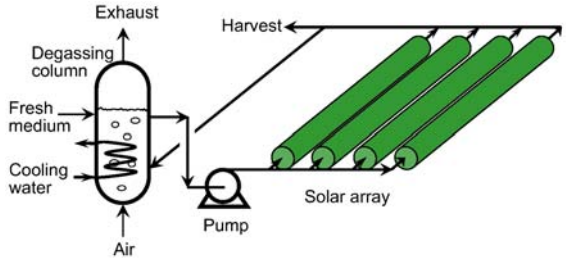

Fig. 2. A tubular photobioreactor with parallel run horizontal tubes.

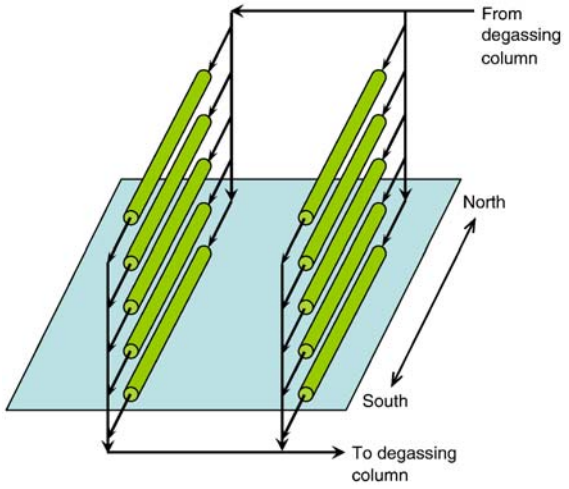

Fig. 3. A fence-like solar collector.

and back to the reservoir. Continuous culture operation is used, as explained above.

The solar collector is oriented to maximize sunlight capture (Molina Grima et al., 1999; Sánchez Mirón et al., 1999). In a typical arrangement, the solar tubes are placed parallel to each other and flat above the ground (Fig. 2). Horizontal, parallel straight tubes are sometimes arranged like a fence (Fig. 3), in attempts to increase the number of tubes that can be accommodated in a given area. The tubes are always oriented North–South (Fig. 3). The ground beneath the solar collector is often painted white, or covered with white sheets of plastic

Fig. 4. A 1000 L helical tubular photobioreactor at Murdoch University, Australia. Courtesy of Professor Michael Borowitzka, Murdoch University.

(Tredici, 1999), to increase reflectance, or albedo. A high albedo increases the total light received by the tubes.

Instead of being laid horizontally on the ground, the tubes may be made of flexible plastic and coiled around a supporting frame to form a helical coil tubular photobioreactors (Fig. 4). Photobioreactors such as the one shown in Fig. 4 are potentially useful for growing a small volume of microalgal broth, for example, for inoculating the larger tubular photobioreactors (Fig. 2) that are needed for producing biodiesel. Other variants of tubular photobioreactors exist (Molina Grima et al., 1999; Tredici, 1999; Pulz, 2001; Carvalho et al., 2006), but are not widely used. Artificial illumination of tubular photobioreactors is technically feasible (Pulz, 2001), but expensive compared with natural illumination. Nonetheless, artificial illumination has been used in large-scale biomass production (Pulz, 2001) particularly for high-value products.

Biomass sedimentation in tubes is prevented by maintaining highly turbulent flow. Flow is produced using either a mechanical pump (Fig. 2), or a gentler airlift pump. Mechanical pumps can damage the biomass (Chisti, 1999a; García Camacho et al., 2001, 2007; Sánchez Mirón et al., 2003; Mazzuca Sobczuk et al., 2006), but are easy to design, install and operate. Airlift pumps have been used quite successfully (Molina Grima et al., 1999, 2000, 2001; Acién Fernández et al., 2001). Airlift pumps for use in tubular photobioreactors are designed using the same methods that were originally developed for designing conventional airlift reactors (Chisti et al., 1988; Chisti and Moo-Young, 1988, 1993; Chisti, 1989). Airlift pumps are less flexible than mechanical pumps and require a supply of air to operate. Periodically, photobioreactors must be cleaned and sanitized. This is easily achieved using automated clean-in-place operations (Chisti and Moo-Young, 1994; Chisti, 1999b).

Photosynthesis generates oxygen. Under high irradiance, the maximum rate of oxygen generation in a typical tubular photobioreactor may be as high as 10 g O_2 m^{-3} min^{-1}. Dissolved oxygen levels much greater than the air saturation values inhibit photosynthesis (Molina Grima et al., 2001). Furthermore, a high concentration of dissolved oxygen in combination with intense sunlight produces photooxidative damage to algal cells. To prevent inhibition and damage, the maximum tolerable dissolved oxygen level should not generally exceed about 400% of air saturation value. Oxygen cannot be removed within a photobioreactor tube. This limits the maximum length of a continuous run tube before oxygen removal becomes necessary. The culture must periodically return to a degassing zone (Fig. 2) that is bubbled with air to strip out the accumulated oxygen. Typically, a continuous tube run should not exceed 80 m (Molina Grima et al., 2001), but the exact length depends on several factors including the concentration of the biomass, the light intensity, the flow rate, and the concentration of oxygen at the entrance of tube.

In addition to removing the accumulated dissolved oxygen, the degassing zone (Fig. 2) must disengage all the gas bubbles from the broth so that essentially bubble-free broth returns to the solar collector tubes. Gas–liquid separator design for achieving complete disengagement of bubbles, has been discussed (Chisti and Moo-Young, 1993; Chisti, 1998). Because a degassing zone is generally optically deep compared with the solar collector tubes, it is poorly illuminated and, therefore, its volume needs to be kept small relative to the volume of the solar collector.

As the broth moves along a photobioreactor tube, pH increases because of consumption of carbon dioxide (Camacho Rubio et al., 1999). Carbon dioxide is fed in the degassing zone in response to a pH controller. Additional carbon dioxide injection points may be necessary at intervals along the tubes, to prevent carbon limitation and an excessive rise in pH (Molina Grima et al., 1999).

Table 3
Comparison of photobioreactor and raceway production methods

Variable	Photobioreactor facility	Raceway ponds
Annual biomass production (kg)	100,000	100,000
Volumetric productivity (kg m^{-3} d^{-1})	1.535	0.117
Areal productivity (kg m^{-2} d^{-1})	0.048 [a] 0.072 [c]	0.035 [b]
Biomass concentration in broth (kg m^{-3})	4.00	0.14
Dilution rate (d^{-1})	0.384	0.250
Area needed (m^2)	5681	7828
Oil yield (m^3 ha^{-1})	136.9 [d] 58.7 [e]	99.4 [d] 42.6 [e]
Annual CO_2 consumption (kg)	183,333	183,333
System geometry	132 parallel tubes/unit; 80 m long tubes; 0.06 m tube diameter	978 m^2/pond; 12 m wide, 82 m long, 0.30 m deep
Number of units	6	8

[a] Based on facility area.
[b] Based on actual pond area.
[c] Based on projected area of photobioreactor tubes.
[d] Based on 70% by wt oil in biomass.
[e] Based on 30% by wt oil in biomass.

Photobioreactors require cooling during daylight hours. Furthermore, temperature control at night is also useful. For example, the nightly loss of biomass due to respiration can be reduced by lowering the temperature at night. Outdoor tubular photobioreactors are effectively and inexpensively cooled using heat exchangers. A heat exchange coil may be located in the degassing column (Fig. 2). Alternatively, heat exchangers may be placed in the tubular loop. Evaporative cooling by water sprayed on tubes (Tredici, 1999), can also be used and has proven successful in dry climates. Large tubular photobioreactors have been placed within temperature controlled greenhouses (Pulz, 2001), but doing so is prohibitively expensive for producing biodiesel.

Selecting a suitable microalgal biomass production method for making biodiesel requires a comparison of capabilities of raceways and tubular photobioreactors.

4. Comparison of raceways and tubular photobioreactors

Table 3 compares photobioreactor and raceway methods of producing microalgal biomass. This comparison is for an annual production level of 100 t of biomass in both cases. Both production methods consume an identical amount of carbon dioxide (Table 3), if losses to atmosphere are disregarded. The production methods in Table 3 are compared for optimal combinations of biomass productivity and concentration that have been actually achieved in large-scale photobioreactors and raceways. Photobioreactors provide much greater oil yield per hectare compared with raceway ponds (Table 3). This is because the volumetric biomass productivity of photobioreactors is more than 13-fold greater in comparison with raceway ponds (Table 3). Both raceway and photobioreactor production methods are technically feasible. Production facilities using photobioreactors and raceway units of dimensions similar to those in Table 3 have indeed been used extensively in commercial operations (Terry and Raymond, 1985; Molina Grima, 1999; Molina Grima et al., 1999; Tredici, 1999; Pulz, 2001; Lorenz and Cysewski, 2003; Spolaore et al., 2006).

Fig. 5. Microalgal biomass recovered from the culture broth by filtration moves along a conveyor belt at Cyanotech Corporation (www.cyanotech.com), Hawaii, USA. Photograph by Terry Luke. Courtesy of Honolulu Star-Bulletin.

Recovery of microalgal biomass from the broth is necessary for extracting the oil. Biomass is easily recovered from the broth by filtration (Fig. 5), centrifugation, and other means (Molina Grima et al., 2003). Cost of biomass recovery can be significant. Biomass recovery from photobioreactor cultured broth costs only a fraction of the recovery cost for broth produced in raceways. This is because the typical biomass concentration that is produced in photobioreactors is nearly 30 times the biomass concentration that is generally obtained in raceways (Table 3). Thus, in comparison with raceway broth, much smaller volume of the photobioreactor broth needs to be processed to obtain a given quantity of biomass.

5. Acceptability of microalgal biodiesel

For user acceptance, microalgal biodiesel will need to comply with existing standards. In the United States the relevant standard is the ASTM Biodiesel Standard D 6751 (Knothe, 2006). In European Union, separate standards exist for biodiesel intended for vehicle use (Standard EN 14214) and for use as heating oil (Standard EN 14213) (Knothe, 2006).

Microalgal oils differ from most vegetable oils in being quite rich in polyunsaturated fatty acids with four or more double bonds (Belarbi et al., 2000). For example, eicosapentaenoic acid (EPA, C20:5*n*-3; five double bonds) and docosahexaenoic acid (DHA, C22:6*n*-3; six double bonds) occur commonly in algal oils. Fatty acids and fatty acid methyl esters (FAME) with 4 and more double bonds are susceptible to oxidation during storage and this reduces their acceptability for use in biodiesel. Some vegetable oils also face this problem. For example, vegetable oils such as high oleic canola oil contain large quantities of linoleic acid (C18:2*n*-6; 2-double bonds) and linolenic acid (C18:3*n*-3; 3-double bonds). Although these fatty acids have much higher oxidative stability compared with DHA and EPA, the European Standard EN 14214 limits linolenic acid methyl ester content in biodiesel for vehicle use to 12% (mol). No such limitation exists for biodiesel intended for use as heating oil, but

acceptable biodiesel must meet other criteria relating to the extent of total unsaturation of the oil. Total unsaturation of an oil is indicated by its iodine value. Standards EN 14214 and EN 14213 require the iodine value of biodiesel to not exceed 120 and 130 g iodine/100 g biodiesel, respectively. Furthermore, both the European biodiesel standards limit the contents of FAME with four and more double bonds, to a maximum of 1% mol.

In view of the composition of many microalgal oils, most of them are unlikely to comply with the European biodiesel standards, but this need not be a significant limitation. The extent of unsaturation of microalgal oil and its content of fatty acids with more than 4 double bonds can be reduced easily by partial catalytic hydrogenation of the oil (Jang et al., 2005; Dijkstra, 2006), the same technology that is commonly used in making margarine from vegetable oils.

6. Economics of biodiesel production

Recovery of oil from microalgal biomass and conversion of oil to biodiesel are not affected by whether the biomass is produced in raceways or photobioreactors. Hence, the cost of producing the biomass is the only relevant factor for a comparative assessment of photobioreactors and raceways for producing microalgal biodiesel.

For the facilities detailed in Table 3, the estimated cost of producing a kilogram of microalgal biomass is $2.95 and $3.80 for photobioreactors and raceways, respectively. These estimates assume that carbon dioxide is available at no cost. The estimation methods used have been described previously (Humphreys, 1991; Molina Grima et al., 2003). If the annual biomass production capacity is increased to 10,000 t, the cost of production per kilogram reduces to roughly $0.47 and $0.60 for photobioreactors and raceways, respectively, because of economy of scale. Assuming that the biomass contains 30% oil by weight, the cost of biomass for providing a liter of oil would be something like $1.40 and $1.81 for photobioreactors and raceways, respectively. Oil recovered from the lower-cost biomass produced in photobioreactors is estimated to cost $2.80/L. This assumes that the recovery process contributes 50% to the cost of the final recovered oil. In comparison with this, during 2006, crude palm oil, that is probably the cheapest vegetable oil, sold for an average price of $465/t, or about $0.52/L.

In the United States during 2006, the on-highway petrodiesel price ranged between $0.66 and $0.79/L. This price included taxes (20%), cost of crude oil (52%), refining expenses (19%), distribution and marketing (9%). If taxes and distribution are excluded, the average price of petrodiesel in 2006 was $0.49/L with a 73% contribution from crude oil and 27% contribution from refining.

Biodiesel from palm oil costs roughly $0.66/L, or 35% more than petrodiesel. This suggests that the process of converting palm oil to biodiesel adds about $0.14/L to the price of oil. For palm oil sourced biodiesel to be competitive with petrodiesel, the price of palm oil should not exceed $0.48/L, assuming an absence of tax on biodiesel. Using the same analogy, a reasonable target price for microalgal oil is $0.48/L for algal diesel to be cost competitive with petrodiesel. Elimination of dependence on petroleum diesel and environmental sustainability require reducing the cost of production of algal oil from about $2.80/L to $0.48/L. This is a strategic objective. The cost reduction necessary declines to $0.72, if the algal biomass is produced in photobioreactors and contains 70% oil by weight. These desired levels of cost reduction are substantial, but attainable.

Microalgal oils can potentially completely replace petroleum as a source of hydrocarbon feedstock for the petrochemical industry. For this to happen, microalgal oil will need to be sourced at a price that is roughly related to the price of crude oil, as follows:

$$C_{\text{algal oil}} = 6.9 \times 10^{-3} C_{\text{petroleum}} \quad (1)$$

where $C_{\text{algal oil}}$ ($ per liter) is the price of microalgal oil and $C_{\text{petroleum}}$ is the price of crude oil in $ per barrel. For example, if the prevailing price of crude oil is $60/barrel, then microalgal oil should not cost more than about $0.41/L, if it is to substitute for crude oil. If the price of crude oil rises to $80/barrel as sometimes predicted, then microalgal oil costing $0.55/L is likely to economically substitute for crude petroleum. Eq. (1) assumes that algal oil has roughly 80% of the energy content of crude petroleum.

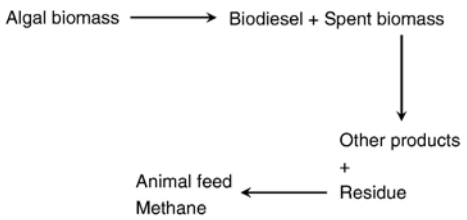

Fig. 6. Microalgal biodiesel refinery: producing multiple products from algal biomass.

7. Improving economics of microalgal biodiesel

Cost of producing microalgal biodiesel can be reduced substantially by using a biorefinery based production strategy, improving capabilities of microalgae through genetic engineering and advances in engineering of photobioreactors.

7.1. Biorefinery based production strategy

Like a petroleum refinery, a biorefinery uses every component of the biomass raw material to produce useable products. Because all components of the biomass are used, the overall cost of producing any given product is lowered. Integrated biorefineries are already being operated in Canada, the United States, and Germany for producing biofuels and other products from crops such as corn and soybean. This approach can be used to reduce the cost of making microalgal biodiesel.

In addition to oils, microalgal biomass contains significant quantities of proteins, carbohydrates and other nutrients (Sánchez Mirón et al., 2003). Therefore, the residual biomass from biodiesel production processes can be used potentially as animal feed (Fig. 6). Some of the residual biomass may be used to produce methane by anaerobic digestion, for generating the electrical power necessary for running the microalgal biomass production facility. Excess power could be sold to defray the cost of producing biodiesel.

Although the use of microalgal biomass directly to produce methane by anaerobic digestion (Mata-Alvarez et al., 2000; Raven and Gregersen, 2007) is technically feasible, it cannot compete with the many other low-cost organic substrates that are available for anaerobic digestion. Nevertheless, algal biomass residue remaining after the extraction of oil can be used potentially to make methane. A microalgal biorefinery can simultaneously produce biodiesel, animal feed, biogas and electrical power (Fig. 6). Extraction of other high-value products may be feasible, depending on the specific microalgae used.

7.2. Enhancing algal biology

Genetic and metabolic engineering are likely to have the greatest impact on improving the economics of production of microalgal diesel (Roessler et al., 1994; Dunahay et al., 1996). Genetic modification of microalgae has received little attention (León-Bañares et al., 2004). Molecular level engineering can be used to potentially:

1. increase photosynthetic efficiency to enable increased biomass yield on light;
2. enhance biomass growth rate;
3. increase oil content in biomass;
4. improve temperature tolerance to reduce the expense of cooling;

Box 2

Light saturation and photoinhibition

Light saturation is characterized by a light saturation constant (Fig. B2), that is the intensity of light at which the specific biomass growth rate is half its maximum value, μ_{max}. Light saturation constants for microalgae tend to be much lower than the maximum sunlight level that occurs at midday. For example, the light saturation constants for microalgae *Phaeodactylum tricornutum* and *Porphyridium cruentum* are 185 $\mu E\ m^{-2}\ s^{-1}$ (Mann and Myers, 1968) and ~200 $\mu E\ m^{-2}\ s^{-1}$ (Molina Grima et al., 2000), respectively. In comparison with these values, the typical midday outdoor light intensity in equatorial regions is about 2000 $\mu E\ m^{-2}\ s^{-1}$. Because of light saturation, the biomass growth rate is much lower than would be possible if light saturation value could be increased substantially.

Above a certain value of light intensity, a further increase in light level actually reduces the biomass growth rate (Fig. B2). This phenomenon is known as *photoinhibition*. Microalgae become photoinhibited at light intensities only slightly greater than the light level at which the specific growth rate peaks. Photoinhibition results from generally reversible damage to the photosynthetic apparatus, as a consequence of excessive light (Camacho Rubio et al., 2003). Elimination of photoinhibition or its postponement to higher light intensities can greatly increase the average daily growth rate of algal biomass.

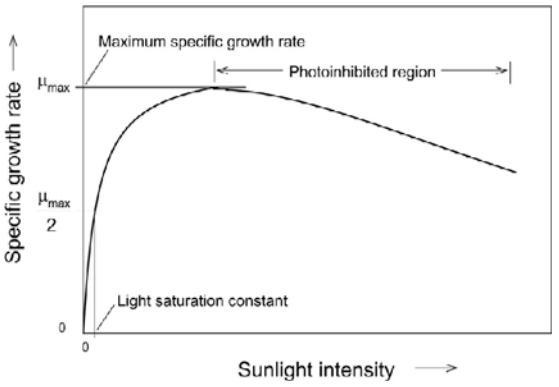

Fig. B2. Effect of light intensity on specific growth rate of microalgae.

5. eliminate the light saturation phenomenon (Box 2) so that growth continues to increase in response to increasing light level;
6. reduce photoinhibition (Box 2) that actually reduces growth rate at midday light intensities that occur in temperate and tropical zones; and
7. reduce susceptibility to photooxidation that damages cells.

In addition, there is a need to identify possible biochemical triggers and environmental factors that might favor accumulation of oil. Stability of engineered strains and methods for achieving stable production in industrial microbial processes are known to be important issues (Zhang et al., 1996), but have been barely examined for microalgae.

7.3. Photobioreactor engineering

Although a capability for reliable engineering and operation of tubular photobioreactors has emerged (Acién Fernández et al., 1997, 1998, 2001; Camacho Rubio et al., 1999; Molina Grima et al., 1999, 2000, 2001; Sánchez Mirón et al., 1999, 2000; Janssen et al., 2003; Carvalho et al., 2006), problems remain.

Photobioreactor tubes operated with high-density culture for attaining high productivity, inevitably contain a photolimited central dark zone and a relatively better lit peripheral zone (Molina Grima et al., 1999, 2001). Light intensity in the photolimited zone is lower than the saturation light level (Box 2). Turbulence in the tube causes rapid cycling of the fluid between the light and dark zones. The frequency of light–dark cycling depends on several factors, including the intensity of turbulence, concentration of cells, optical properties of the culture, the diameter of the tube, and the external irradiance level (Molina Grima et al., 2000, 2001). Under conditions of sufficient and excess external irradiance, light–dark cycling of above a certain frequency can increase biomass productivity relative to the case when the same quantity of light is supplied continuously over the same total exposure time (Philliphs and Myers, 1953; Terry, 1986; Grobbelaar, 1994; Nedbal et al., 1996; Grobbelaar et al., 1996; Camacho Rubio et al., 2003). Light–dark cycling times of 10 ms, for example, are known to improve growth compared with continuous illumination of equal cumulative quantity. Beneficial effects of rapid light–dark cycling under light saturation conditions are associated with the short dark period allowing the photosynthetic apparatus of the cells to fully recover from the excited state of the previous illumination event.

Various attempts have been made to estimate the frequency of light–dark cycling (Molina Grima et al., 1999, 2000, 2001; Sánchez Mirón et al., 1999; Janssen et al., 2003; Richmond, 2004), but this problem remains unresolved. Distinct from the productivity enhancing effect of light–dark cycling, turbulence in a dense culture reduces photoinhibition and photolimitation by ensuring that the algal cells do not reside continuously in either the well lit zone or the dark zone for long periods.

In principle, motionless mixers installed inside photobioreactor tubes can be used to substantially enhance the mixing between the peripheral lit zone and the interior dark zone (Molina Grima et al., 1999, 2001; Sánchez Mirón et al., 1999). Such mixers have proved useful in other tubular reactors (Chisti et al., 1990; Chisti, 1998; Thakur et al., 2003). Unfortunately, existing designs of motionless mixers are not satisfactory for photobioreactors because they substantially reduce penetration of light in the tubes. New designs of motionless mixers are needed.

Like cells of higher plants (Moo-Young and Chisti, 1988) and animals (Zhang et al., 1995; Chisti, 2000, 2001; García Camacho et al., 2005), microalgae are damaged by intense hydrodynamic shear fields that occur in high-velocity flow in pipes, pumps and mixing tanks (Chisti, 1999a; García Camacho et al., 2001, 2007; Sánchez Mirón et al., 2003; Mazzuca Sobczuk et al., 2006). Some algae are more sensitive to shear damage than others. Shear sensitivity can pose a significant problem as the intensity of turbulence needed in photobioreactors to generate optimal light–dark cycling (Grobbelaar et al., 1996; Camacho Rubio et al., 2003) is difficult to achieve (Molina Grima et al., 2000, 2001; Camacho Rubio et al., 2004) without damaging algal cells. Methods have been developed to reduce the damage associated with turbulence of limited intensity (García Camacho et al., 2001; Mazzuca Sobczuk et al., 2006). Intensities of shear stress are not easily determined in bioreactors (Chisti and Moo-Young, 1989; Chisti, 1989, 1999a), but improved methods for doing so are emerging (Sánchez Pérez et al., 2006).

Some algae will preferentially grow attached to the internal wall of the photobioreactor tube, thus preventing light penetration into the tube and reducing bioreactor productivity. Robust methods for controlling wall growth are needed. Wall growth is controlled by some of the following methods: 1. use of large slugs of air to intermittently scour the internal surface of the tube; 2. circulation of close fitting balls in continuous run tubes to clean the internal surface; 3. highly turbulent flow; and 4. suspended sand or grit particles to abrade any biomass adhering to the internal surface. Potentially, enzymes that

digest the polymer glue that binds algal cells to the tube walls, may be used for controlling wall growth.

Bioprocess intensification approaches (Chisti and Moo-Young, 1996; Chisti, 2003) that have proved so successful in improving the economics of various biotechnology based processes have been barely assessed for use with photobioreactors.

8. Conclusion

As demonstrated here, microalgal biodiesel is technically feasible. It is the only renewable biodiesel that can potentially completely displace liquid fuels derived from petroleum. Economics of producing microalgal biodiesel need to improve substantially to make it competitive with petrodiesel, but the level of improvement necessary appears to be attainable. Producing low-cost microalgal biodiesel requires primarily improvements to algal biology through genetic and metabolic engineering. Use of the biorefinery concept and advances in photobioreactor engineering will further lower the cost of production. In view of their much greater productivity than raceways, tubular photobioreactors are likely to be used in producing much of the microalgal biomass required for making biodiesel. Photobioreactors provide a controlled environment that can be tailored to the specific demands of highly productive microalgae to attain a consistently good annual yield of oil.

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